

CLAIMS

1. An oligonucleotide,
 - (a) wherein the third nucleotide from the 3'-end thereof is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides; and
 - (b) wherein the oligonucleotide has a nucleotide complementary to the reference nucleotide of a target gene at the 3'-end position thereof, and has nucleotides complementary to the nucleotide sequence of the target gene at the other positions, or a salt thereof.
2. An oligonucleotide,
 - (a) wherein the third nucleotide from the 3'-end thereof is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides; and
 - (b) wherein the oligonucleotide has a nucleotide complementary to the mutant nucleotide of a target gene at the 3'-end position thereof, and has nucleotides complementary to the nucleotide sequence of the target gene at the other positions, or a salt thereof.
3. An oligonucleotide,
 - (a) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the reference nucleotide of a target gene;

(b) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(c) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

(d) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof.

4. An oligonucleotide,

(a) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the mutant nucleotide of a target gene;

(b) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(c) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

(d) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof.

5. An oligonucleotide or a salt thereof according to any one of claims 1 to 4, characterized by having a base length of 18 to 25 bases.

6. A method for detecting gene polymorphism, characterized by using an oligonucleotide according to any one of claims 1 to 5.

7. A method for determining the nucleotide sequence of a genetically polymorphic sequence, characterized by using an oligonucleotide according to any one of claims 1 to 5.

8. A method for detecting gene polymorphism, comprising the following steps (a) and (b):

(a) a step of performing PCR with nucleic acid comprising a genetically polymorphic sequence as a template using an oligonucleotide according to any one of claims 1 to 5 and an oligonucleotide capable of amplifying a sequence of interest together with said oligonucleotide in the PCR; and

(b) a step of determining the presence or absence of gene polymorphism in the nucleic acid based on whether or not a reaction product can be generated in step (a).

9. A method for determining the nucleotide sequence of a genetically polymorphic sequence, comprising the following steps (a) and (b):
- (a) a step of performing PCR with nucleic acid comprising a genetically polymorphic sequence as a template using an oligonucleotide according to any one of claims 1 to 5 and an oligonucleotide capable of amplifying the sequence of interest together with said oligonucleotide in the PCR; and
- (b) a step of determining the nucleotide sequence of a genetically polymorphic sequence in the nucleic acid based on whether or not a reaction product can be generated in step (a).
10. A method according to claim 8 or 9, characterized by using, for detection of the presence or absence of generation of a reaction product, one or more method selected from the group consisting of electrophoresis, TaqMan PCR, and a MALDI-TOF/MS method.
11. A method according to any one of claims 6 to 10, characterized in that the gene polymorphism is a single nucleotide polymorphism.
12. A kit for detecting gene polymorphism, which comprises the following (a) to (d):
- (a) an oligonucleotide, wherein the third nucleotide from the 3'-end thereof is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, the other nucleotides are natural nucleotides,

the 3'-end nucleotide thereof is a nucleotide complementary to the reference nucleotide of a target gene, and at the other positions the nucleotides are complementary to the nucleotide sequence of the target gene;

(b) an oligonucleotide capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer.

13. A kit for detecting gene polymorphism, comprising the following (a) to (d):

(a) an oligonucleotide, wherein the third nucleotide from the 3'-end thereof is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, the other nucleotides are natural nucleotides, the 3'-end nucleotide thereof is a nucleotide complementary to the mutant nucleotide of a target gene, and at the other positions the nucleotides are complementary to the nucleotide sequence of the target gene;

(b) a primer capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer.

14. A kit for detecting gene polymorphism, comprising the following (a) to (e):

- (a) an oligonucleotide, wherein the third nucleotide from the 3'-end thereof is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, the other nucleotides are natural nucleotides, the 3'-end nucleotide thereof is a nucleotide complementary to the reference nucleotide of a target gene, and at the other positions the nucleotides are complementary to the nucleotide sequence of the target gene;
- (b) an oligonucleotide, wherein the third nucleotide from the 3'-end thereof is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, the other nucleotides are natural nucleotides, the 3'-end nucleotide thereof is a nucleotide complementary to the mutant nucleotide of a target gene, and at the other positions the nucleotides are complementary to the nucleotide sequence of the target gene;
- (c) an oligonucleotide capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) or (b) above;
- (d) DNA polymerase; and
- (e) a PCR buffer.

15. A kit for detecting gene polymorphism, comprising the following (a) to (d):

- (a) an oligonucleotide,
 - (i) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the reference nucleotide of a target gene;

(ii) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(iii) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

(iv) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof;

(b) an oligonucleotide capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer.

16. A kit for detecting gene polymorphism, comprising the following (a) to (d):

(a) an oligonucleotide,

(i) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the mutant nucleotide of a target gene;

(ii) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(iii) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

(iv) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides; or a salt thereof;

(b) an oligonucleotide capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer.

17. A kit for detecting gene polymorphism, comprising the following (a) to (e):

(a) an oligonucleotide having the following characteristics (i) to (iv)

(i) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the reference nucleotide of a target gene;

(ii) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(iii) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

(iv) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof;

(b) an oligonucleotide,

(i) wherein the 3'-end nucleotide thereof is a nucleotide complementary to the mutant nucleotide of a target gene;

(ii) wherein the second nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a nucleotide that is not complementary to the nucleotide of a reference gene;

(iii) wherein the oligonucleotide has nucleotides complementary to the nucleotides of the target gene at the other positions; and

- (iv) wherein the third nucleotide from the 3'-end thereof (when the nucleotide at the 3'-end is defined as the first nucleotide) is a 2'-*O*,4'-*C*-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof;
- (c) an oligonucleotide capable of amplifying a sequence of interest, together with the oligonucleotide described in (a) or (b) above;
- (d) DNA polymerase; and
- (e) a PCR buffer.
18. A kit for detecting gene polymorphism according to any one of claims 12 to 17, characterized in that the oligonucleotide, and the oligonucleotide capable of amplifying a sequence of interest together with said oligonucleotide, each have a base length of 18 to 25 bases.
19. A kit according to any one of claims 12 to 18, characterized in that the gene polymorphism is a single nucleotide polymorphism.